QuickBlue

bioanalytic

Campylobacter jejuni

Real-Time PCR DNA Detection Kit

User manual

www.QuickBlue.de

For food testing only

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Substances	QB-RT-01-50	QB-RT-01-250	QB-RT-01-1000
QuickBlue DNA Detection Kit Components for 25 µl reaction	50	250	1000
1. PCR premaster (white) contains: •RNase free water • PCR buffer • MgCl2 (1,5 mM) • dNTP-Mix including dUTP • primer pair • HotMaster Taq polymerase	1 Cup	5 Cups	10 Cups
2. Uracil-Glycosylase (UNG) (yellow)	1 Cup	1 Cup	1 Cup
3. TaqMan probe (green)	1 Cup	1 Cup	1 Cup
4. Reference DNA (positive control) (red)	1 Cup	1 Cup	1 Cup
5. RNase free water (blue)	1 Cup	2 Cups	5 Cups
6. User manual	1	1	1

Additional required material:

Instrumentation and media for preculture (if necessary), equipment for water-filtration (if necessary), PBS buffer, DNA extraction kits, Instruments for PCR (Realtime cycler).

Storage:

The QuickBlue DNA Detection PCR Kit should be stored at - 20 °C , short storage at + 5 °C is possible. After thawing the Premaster shall be stored at +4 °C!
Correct storage and handling increases the stability of the PCR kit!

Overview PCR detection:

If applicable: preculture (A) / water-filtration (B)

(A) Pre-enrichment of sample in an appropriate (selective) medium (1:10 w/v: 25 g sample + 225 ml medium) for 20-24 hours at appropriate temperature.
(B) Filtration of 100 ml with an appropriate filter system* (0,45 µm!), e.g. cellulose nitrate filter.

Bacteria DNA preparation

(A) Use QuickBlue DNA purification kit or other appropriate DNA purification methods. Thermal lysis may only be used in matrices which does not contain components inhibiting the PCR reaction.

QuickBlue-Kit

DNA purification

bacteria: (A) after DNA preparation dilute DNA 1:10 in TE buffer to avoid PCR inhibition.

(B) extract DNA from the filter directly (e.g. with CTAB lysis buffer*);
eucaryota: use a commercial DNA purification kit* like column systems or CTAB extraction.

Mastermix preparation

combine premaster (white), TaqMan probe (green) and UNG (yellow), amounts see table 3 (page 9), mix well (= Mastermix).

PCR pipetting

Pipetting: 24 µl Mastermix
and 1 µl DNA
or 1 µl H_2O (= negative control, blue)
or 1 µl reference DNA (= positive control, red)

Thermocycling

Initialphase:	2 min	50 °C, 1 min 95 °C,
45 cyles:	20 s	95 °C,
	30 s	60 °C,
	20 s	72 °C
End phase:	hold	10 °C

* not included in the PCR kit

Specifications

Specificity
Campylobacter jejuni

The primer and probe systems are species specific for the respective **QuickBlue** PCR DNA Detection Kits.

Sensitivity
After pre-enrichment < 1 cells / 25 g
Without pre-enrichment < 10 cells / g or 10 non-degraded genomes

Technical support
The Q-Bioanalytic GmbH offers a qualified technical support. If you have questions or problems with the handling of the PCR detection kit, do not hesitate to contact us. For our technical support and more information about our QuickBlue PCR Detection Kit please call phone.: +49 - (0)471 - 48 32 440.

Product limitations
QuickBlue DNA Detection Kit is licensed for food testing only.

Safety information
When working with the QuickBlue DNA PCR Detection Kit, protective clothing should be worn as well as lab gloves. Work should be done under PCR-sterile conditions. Avoid contact with DNA or DNA contaminated areas and surfaces during PCR mastermix preparation. First pipette the mastermix and subsequently the DNA into the PCR tubes or PCR plates.

Introduction

Target organisms will be detected with species specific oligonucleotides (specific primer from German LFGB § 64 or NCBI database entries). High specificity and sensitivity of the PCR kit result from chosen PCR components as well as special Taq-Enzyme and PCR buffer. The QuickBlue DNA Detection PCR Kit was optimized on several thermocyclers. The user manual contains a general protocol for this PCR application. Kits were validated with Roche LightCycler 480, Applied Biosystems StepOne, Stratagene MX 4000 and Eppendorf RealPlex Cyclers.

Protocol

After appropriate pre-enrichment and/or DNA preparation perform the following steps for the PCR:

- Preparation of mastermix
- Pipetting the samples and controls
- Thermocycling
- Evaluation

Important advices for mastermix preparation

- Start the PCR only with the PCR program as shown in table 1 (page 6)
- Components are optimized for a volume of 25 µl
- Pipette always 1 µl of your DNA sample to the specific mastermix to a total volume of 25 µl
- Use only declared PCR tubes and PCR plates (from polyethylen, polypropylene or polycarbonate, no polystyrene!!!) for this PCR kit.

Mastermix preparation

Prearrangement
The PCR components have to thaw in a sterile, DNA-free and PCR product free area (→ separate PCR room or PCR hood). Place the pipettes, tips and waste container closely to your workbench so you could not touch other objects with your disposable lab gloves during pipetting. This is to avoid contaminations of your PCR components. Set the PCR tubes/plate and the DNA samples in a separate laboratory or aside the PCR hood.

PCR program
Before pipetting make sure that following PCR program is on your thermo-cycler:

Table 1: Demonstration of the specific PCR program

PCR steps	Time	Temperature	Commentary
UNG activity	2 min	50 °C	eliminates contaminations
Initial denaturation	1 min	95 °C	activates HotStart enzyme
Number of cycles		**x 45**	defines product amount
Denaturation	20 s	95 °C	denaturation of dsDNA
Primer annealing	30 s	60 °C	primer annealing on ssDNA, fluorescence measurement (6-Fam filter system, 483 nm)
Elongation	20 s	72 °C	primer extension to PCR product
storage	hold	10 °C	storage temperature

Pipetting the PCR mastermix and DNA samples

Table 2: Demonstration of the composition (per PCR reaction)

Components	**Volume / reaction**	**End concentration**
RT-PCR premaster (white)	12,5 µl	-
RNase free water (blue)	11,0 µl	-
UNG (yellow)	0,2 µl	0,2 Unit
Probe (green)	1,0 µl	
Mix volume	**24,7 µl**	
DNA	1,0 µl	variable
Total volume	**25,7 µl**	**-**

1. Depending on your total PCR amount pipette the adequate volumes of premaster, water, Uracil-Glycosylase (UNG) and probe in a sterile PCR tube (e.g.: 1,5-ml reaction cup).

2. Mix the mastermix on a shaker, to disperse components homogeneously.

3. Put the PCR components back into the freezer (at - 20 °C).

4. Aliquot 24 µl of your mastermix into the labeled PCR tubes (0,2 ml tubes, 8-module-strips, or 96/384-PCR-plate). Don't forget controls!

5. Pipette 1 µl of your sample DNA per cavity into the mastermix. This avoids crosscontaminations!

6. Don't forget to pipette 1 µl of the negative control (blue cup) and 1 µl of the positive control (red cup) into separate PCR mastermixes. Include a positive control reaction in your DNA preparation to exclude effects of inhibition by the matrix analysed.

7. Close the tubes or plates well and put them into the thermocycler.

Thermocycling

Start the specific PCR program (see table 1, page 6).

Table 3: Preparation of premaster for parallel approaches:

Number:	H_2O:	Premaster:	Probe:	UNG:	Number:	H_2O:	Premaster:	Probe:	UNG:
1	11	12.5	1	0.2	49	539	612.5	49	9.8
2	22	25	2	0.4	50	550	625	50	10
3	33	37.5	3	0.6	51	561	637.5	51	10.2
4	44	50	4	0.8	52	572	650	52	10.4
5	55	62.5	5	1	53	583	662.5	53	10.6
6	66	75	6	1.2	54	594	675	54	10.8
7	77	87.5	7	1.4	55	605	687.5	55	11
8	88	100	8	1.6	56	616	700	56	11.2
9	99	112.5	9	1.8	57	627	712.5	57	11.4
10	110	125	10	2	58	638	725	58	11.6
11	121	137.5	11	2.2	59	649	737.5	59	11.8
12	132	150	12	2.4	60	660	750	60	12
13	143	162.5	13	2.6	61	671	762.5	61	12.2
14	154	175	14	2.8	62	682	775	62	12.4
15	165	187.5	15	3	63	693	787.5	63	12.6
16	176	200	16	3.2	64	704	800	64	12.8
17	187	212.5	17	3.4	65	715	812.5	65	13
18	198	225	18	3.6	66	726	825	66	13.2
19	209	237.5	19	3.8	67	737	837.5	67	13.4
20	220	250	20	4	68	748	850	68	13.6
21	231	262.5	21	4.2	69	759	862.5	69	13.8
22	242	275	22	4.4	70	770	875	70	14
23	253	287.5	23	4.6	71	781	887.5	71	14.2
24	264	300	24	4.8	72	792	900	72	14.4
25	275	312.5	25	5	73	803	912.5	73	14.6
26	286	325	26	5.2	74	814	925	74	14.8
27	297	337.5	27	5.4	75	825	937.5	75	15
28	308	350	28	5.6	76	836	950	76	15.2
29	319	362.5	29	5.8	77	847	962.5	77	15.4
30	330	375	30	6	78	858	975	78	15.6
31	341	387.5	31	6.2	79	869	987.5	79	15.8
32	352	400	32	6.4	80	880	1000	80	16
33	363	412.5	33	6.6	81	891	1012.5	81	16.2
34	374	425	34	6.8	82	902	1025	82	16.4
35	385	437.5	35	7	83	913	1037.5	83	16.6
36	396	450	36	7.2	84	924	1050	84	16.8
37	407	462.5	37	7.4	85	935	1062.5	85	17
38	418	475	38	7.6	86	946	1075	86	17.2
39	429	487.5	39	7.8	87	957	1087.5	87	17.4
40	440	500	40	8	88	968	1100	88	17.6
41	451	512.5	41	8.2	89	979	1112.5	89	17.8
42	462	525	42	8.4	90	990	1125	90	18
43	473	537.5	43	8.6	91	1001	1137.5	91	18.2
44	484	550	44	8.8	92	1012	1150	92	18.4
45	495	562.5	45	9	93	1023	1162.5	93	18.6
46	506	575	46	9.2	94	1034	1175	94	18.8
47	517	587.5	47	9.4	95	1045	1187.5	95	19
48	528	600	48	9.6	96	1056	1200	96	19.2

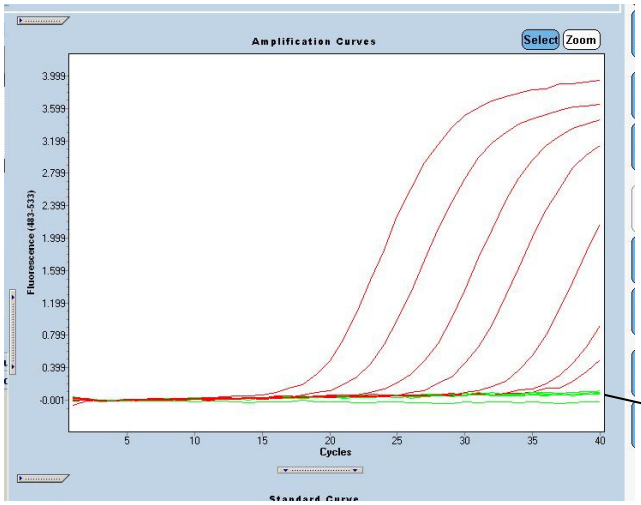

Figure 2: Demonstration of RT-PCR results performed with a LightCycler ® 480 instrument. A serial dilution with reference DNA (curve 1 – 7) and the negative controls is shown.

The curves no 1 - 7 show a dilution series of *Cmpylobacter jejuni* DNA. The bottom lines are negative controls and DNA of non target species.

The first amplification signal can appear with the 14th cycle. This depends on the purity of DNA (→ inhibition by matrix components). Signals after cycle 38 should be repeated with higher concentrated sample.

Trouble Shooting

Problem	Comments and suggestions
No flourescence with the positive control	Pipetting failure, positive DNA degraded, probe missing
PCR product in negative control	UNG missing, pipetting failure with genomic sample DNA, PCR premaster or mastermix contaminated during preparation
To little amount of components in the PCR Kit	Pipetting failure in previous PCR preparations, so the volumes of the components will not suffice
No curves	Wrong filter system: 6-Fam filter systems were not chosen

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QuickBlue RealQuick PCR DNA Detection Kits

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